CERTIFICATE OF VERIFICATION

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state that the attached document is a true and complete translation to the best of my knowledge of German priority document DE 199 37 264.0.

Dated: July 4, 2006

Signature of Translator: Ursula Scherz

URSULA SCHERZ

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The attached sheets are true and exact reproductions of the original documents of this patent application.

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K 2839

F_V Antibody Constructs

The present invention relates to F_V antibody constructs which can induce a regression of Hodgkin's disease, to DNAs coding for such F_V antibodies and a method of producing the F_V antibody constructs as well as their use.

Natural antibodies have four variable domains, *i.e.* two V_H domains and two V_L domains. The variable domains serve as binding sites for an antigen, a binding site being formed of a V_H domain and a V_L domain. Natural antibodies have two equal binding sites, *i.e.* they recognize an antigen and are therefore also referred to as being monospecific. Artificial antibodies may also have two different binding sites, *i.e.* they then recognize two antigens and are correspondingly referred to as being bispecific. An example of such antibodies is an antibody recognizing the FcγIIIA receptor (CD16) of natural killer cells (NK cells) and of the surface protein CD30 of Hodgkin's disease cells. NK cells can be activated by this antibody (bimAbHRS-3/A9) and directed against Hodgkin's disease cells so as to induce a regression of Hodgkin's disease (cf. Hartmann, F *et al.*, Blood 89 (1997), 2042). On the other hand, it turned out that bimAbHRS-3/A9 can only be produced or purified with difficulty. It also turned out that bimAbHRS-3/A9 causes undesired immune responses in many patients.

It is thus the object of the present invention to provide an antibody serving for inducing a regression of Hodgkin's disease by avoiding the above drawbacks.

According to the invention this is achieved by the subject matters defined in the claims.

The present invention is based on Applicant's insights that an F_v antibody construct comprising binding sites for a CD16 receptor and a CD30 surface protein can induce a regression of Hodgkin's disease, the lysis of the tumor cells being more intense than when bimAbHRS-3/A9 is used. He also found out that such an F_v antibody construct can be produced in large amounts and with high purity. Furthermore, the F_v antibody construct distinguishes itself in that it contains no portions which can result in undesired immune responses in patients.

According to the invention Applicant's insights are used to provide an F_v antibody construct having binding sites for a CD16 receptor and a CD30 surface protein.

The term "F_v antibody construct" refers to an antibody construct having variable domains but no constant domains. In particular, bindings sites for a CD16 receptor and a CD30 surface protein are available as variable domains.

The expression "binding site" refers to a V_H domain and a V_L domain by means of which the F_v antibody construct may bind to a CD16 receptor and/or a CD30 surface protein.

The term "CD16 receptor" comprises a CD16 receptor of any kind and origin. For example, the CD16 receptor may be derived from NK cells, macrophages or activated monocytes. The CD16 receptor may also be present in wild-type or modified form, the latter form also comprising a fragment of a CD16 receptor to which an antibody directed against a CD16 receptor can bind.

The expression "CD30 receptor" comprises a CD30 receptor of any kind and origin. For example, the CD30 receptor may be derived from Hodgkin's disease cells or Reed-Sternberg cells. The CD30 receptor may also be available in wild-type or modified form, the latter form also comprising a fragment of a CD30 receptor to which an antibody directed against a CD30 receptor can bind.

An F_V antibody construct according to the invention has one or more binding sites for a CD16 receptor and one or more binding sites for a CD30 surface protein. The F_V antibody construct preferably has one or two binding sites for a CD16 receptor and one or two binding sites for a CD30 surface protein.

An F_V antibody construct according to the invention can also be prepared by various methods. For example, an F_V antibody construct which has a binding site for a CD16 receptor and a binding site for a CD30 surface protein, can be produced e.g. by expressing a first single-chain F_V antibody construct which has a V_H domain of an anti-CD16 antibody and a V_L domain of an anti-CD30 antibody, together with a second single-chain F_V antibody construct which has a V_L domain of an anti-CD16 antibody and a V_H domain of an anti-CD30 antibody, so that both are placed together and the F_V antibody construct according to the invention is formed. Reference is made to Example 1-3 by way of supplement.

An F_V antibody construct which has two to four binding sites for a CD16 receptor and two binding sites for a CD30 surface protein, can also be produced e.g. by expressing a single-chain F_V antibody construct comprising the elements (a) and (b):

(a) a V_H domain of an anti-CD16 antibody and a V_L domain of an anti-CD30 antibody, the domains being joined via a peptide linker 1 which may comprise any amino acids, in particular glycine (G), serine (S) and proline (P), and preferably 0 to 10 amino acids,

(b) a V_H domain of an anti-CD30 antibody and a V_L domain of an anti-CD16 antibody, the domains being joined via the above peptide linkers 1,

the elements (a) and (b) being joined via a peptide linker 2 which may comprise any amino acids, in particular glycine, serine and proline, and preferably 3 to 10 amino acids, and more preferably the amino acid sequence GGPGS. Reference is made to Applicant's patent application 198 19 846.9 by way of supplement.

Another subject matter of the present invention relates to a nucleic acid, in particular a DNA, which codes for an above F_V antibody construct. Furthermore, expression vectors containing such a DNA also represent a subject matter of the present invention. The preferred expression vector is pKID16-30 of figure 1. It was deposited with DSMZ (*Deutsche Sammlung für Mikroorganismen und Zellen* [German-type collection for micro-organisms and cells]) under DSM 12960 on July 29, 1999. Cells containing an above expression vector are also a subject matter of the present invention.

Another subject matter of the present invention is a kit comprising

(a) an F_V antibody construct according to the invention, and/or

(b) an expression vector according to the invention, as well as

(c) common auxiliary substances, such as buffers, solvents, carriers, controls and markers.

One or more representatives of the individual components may be present.

The present invention provides an F_V antibody construct having binding sites for a CD16 receptor and a CD30 surface protein. This F_V antibody construct can be produced in large amounts and with high purity. Also, it does not contain any portions or fragments which can result in undesired immune responses in patients. The F_V antibody construct distinguishes itself in particular by activating NK cells and adjusting or orienting them to cells expressing CD30 surface proteins, in particular tumor cells, more preferably Hodgkin's disease cells or Reed-Sternberg cells, so as to lyse these cells. Thus, the present invention is suited to take steps against diseases in which cells expressing CD30 surface proteins play a role. Such diseases are e.g. tumoral diseases, in particular Hodgkin's disease.

Brief description of the drawings:

Figure 1 shows the pKID16-30 expression vector according to the invention. It codes for two single-chain F_V antibody constructs one of which having the V_H domain of an anti-CD16 antibody and the V_L domain of an anti-CD30 antibody and the other having the V_H domain of an anti-CD30 antibody and the V_L domain of an anti-CD16 antibody. Having expressed the single-chain F_V antibody constructs, they are

placed together so as to obtain the F_v antibody construct according to the invention.

Figure 2 shows a FACS analysis of the binding of an F_v antibody construct according to the invention to $CD30^+$ L540CY Hodgkin's disease cells and $CD16^+$ granulocytes. The tumor cells and the granulocytes were incubated with 20 µg of the F_v antibody construct according to the invention each. The binding of the F_v antibody construct was determined using the anti-c-myc antibody 9E10 and fluorescein-conjugated goat anti-mouse IgG. The cells were incubated as such with 9E10 and fluorescein-conjugated goat anti-mouse IgG as negative control.

Figur 3 shows the cytolytic activity of NK cells contained in peripheral blood lymphocytes (PBL cells) (effector) as against $CD30^+$ L540CY Hodgkin's disease cells (target cells) with different effector target cell relationships in a 5 h JAM test. An F_v antibody construct according to the invention (●) was administered at a concentration of 1 µg/ml. bimAbHRS-3/A9 (▲) was used at a concentration of 4 µg/ml as a control. The F_v antibody construct without NK cells (o) and NK cells alone (□) were used as negative control.

Figure 4 shows the treatment of SCID mice carrying Hodgkin's disease xenografts with an F_v antibody construct according to the invention. On day 0, the mice were treated i.v. using 100 µg of an F_v antibody construct according to the invention together with PBL cells containing NK cells (●) or

without them (o), with 200 µl PBS (*), with 1 x 10^7 PBL cells (□) or with a mixture of 100 µg mAb HRS-3 and A9 together with PBL cells (◇). The tumor diameters were measured twice a week, and the tumor volume was calculated using the following formula: volume = $d^2 x\ Dx\pi/6$, d being the smaller tumor diameter and D being the larger one.

Example 1: Construction of the pKID16-30 expression vector according to the invention

The cDNA of the V_H and V_L domains of an anti-CD16 antibody mAb A9 was subjected to PCR. The following primers were used for this purpose:

VH5', 5-CAGCCGGCCATGGCGCAGGTC(G)CAGCTGCAGC(G)AG-3 (NcoI);

VH3', 5-CCAGGGGCCAGTGGATAGACAAGCTTGGGTGTTGTTTT-3 (HindIII);

VL5', 5-AGAGACGCGTACAGGCTGTTGTGACTCAGG-3 (MluI);

VL3', 5-GACTGCGGCCGCAGACTTGGGCTGGCC-3 (NotI).

The PCR was carried out as follows: one cycle; 5 min. at 94°C, 3 min. at 58°C, and 2 min. at 72°C, followed by 30 cycles; 80 sec. at 94°C, 80 sec. at 58°C, and 2 min. at 72°C and/or the latter 10 min. in the last cycle. The PCR products were gel-purified and inserted in the pCR-Script SK(+) vector (Stratagene company) for sequencing. For the purpose of expression the V_H domain was inserted via NcoI/HindIII and the V_L domain was inserted via MluI/NotI in the pHOG21 vector.

The V_H and V_L domains of an anti-CD30-scF_v fragment were subjected to PCR. The following primers were used for this:

5-ATGACCATGATTACGCCAAGC-3

5-AGACAAGCTTGGGTGTTGTTTTGGCTGAGGAGACGG-3 (HindIII);

5-GGCGGATATCGAGCTCACTCAGTCTCC-3 (EcoRV)

5-TATAGCGGCCGCAGCATCAGCCCGTTTGATTTCC-3 (NotI).

The V_H and V_L domains of the anti-CD30-scFv fragment or the anti-CD16-scFv fragment were inserted in the expression vector pKID so as to obtain the pKID 16-30 expression vector according to the invention. It codes for the single-chain F_V antibody constructs V_H, 16-V_L 30 and V_H 30-V_L 16.

Example 2: Expression of the F_V antibody construct according to the invention in bacteria

E. coli-X11 Blue cells (Stratagene, La Jolla, C.A.) which had been transformed with the pKID16-30 expression plasmid, were cultured in 2YT medium using 100 µg/ml ampicillin and 100 mM glucose at 37°C overnight. 1:20 dilutions of the overnight cultures were cultured as flask cultures in 2YT medium at 38°C while shaking at 280 rpm. The bacteria were pelleted by 10-minute centrifugation at 1500 g at 20°C with an OD_{600} value of 0.8 and resuspended in the same volume of a fresh 2YT medium containing 100 µg/ml ampicillin and 0.4 M saccharose. IPTG was added at a final concentration of 0.1 M and the growth was continued at 21°C (20-22°C) for 18-20 h. The F_V antibody construct was isolated as described by Kipriyanov, S.M. *et al.*, Protein Engineering 10, (1997), 445. Thereafter, it was concentrated by ammonium sulfate precipitation (final concentration: 70 % saturation). The protein precipitate was obtained by centrifugation (30,000 g, 4°C, 45 min.) and dissolved in 10 % of the initial volume from 50 mM Tris-HCl, 1 M NaCl, pH 7.0. An immobilized metal affinity chromatography (IMAC) was carried out as described by Kipriyanov, S.M. *et al.*, J. Immunol. Methods 200, (1997), 69. The purified F_V antibody construct was dialyzed against a phosphate-buffered common salt solution.

Example 3: Characterization of the F_V antibody construct according to the invention

(A) Flow cytometry

In order to detect the binding of an F_V antibody construct according to the invention to $CD16^+$ granulocytes and $CD30^+$ L540CY Hodgkin's disease cells, FACScan (Beckton Dickinson) analysis was carried out. For this purpose, 1×10^6 cells were washed twice in icecold PBS-N (PBS, 0.05 % NaN_3) and incubated on ice with 100 µl of the F_V antibody construct of Example 2 for 45 min. The cells were pelleted at 1200 rpm at 4°C for 5 min. and washed with 2 ml PBS-N. The cells were resuspended in 100 µl PBS-N containing 10 µg/ml of the 9E10 antibody binding to c-myc (ICI Chemikalien), and incubated on ice for 30 min. The cells were pelleted and washed as described above. Thereafter, the cells were resuspended with fluorescein-labeled goat anti-mouse IgG (Gibco BRL; diluted 1:100 in PBS-N), and incubated on ice for 30 min. After another wash step using PBS-N, the cells were ready for analysis with PBS-N containing 1 µg/ml propidium iodide (Sigma). Background fluorescence was determined by incubating the cells with the 9E10 antibody and fluorescein-labeled goat anti-mouse IgG under equal conditions.

It turned out that the F_V antibody construct according to the invention detects both $CD16^+$ granulocytes and $CD30^+$ L540 CY Hodgkin's disease cells and binds to them.

(B) Cytotoxicity assay

In order to detect the activity of an F_V antibody construct according to the invention regarding the activation of NK cells and the lysis of $CD30^+$ L540CY Hodgkin's disease cells,

a cytotoxicity test was carried out according to the JAM test described by Matzinger, P., J. Immunol. Meth. 145 (1991), 185. DNA fragmentation is evaluated in the cytotoxicity test. Cells were labeled using [^{3}H] thymidine up to a final concentration of 2.5-5 µCi/(ml for 4-6 h. The cells were pelleted, washed once with culture broth and adjusted to 10^4 cells/well of a 96-well plate. Having added effector cells (peripheral blood cells containing NK cells ("PBL cells") at various dilutions, the 96-well plate was incubated in a humidified atmosphere with 7.5 % CO_2 for 4 h. The cells and the medium were sucked onto fiber glass filters. Having washed and dried the filters, they were transferred into plastic bags containing a scintillation liquid and counted using a liquid scintillation counter (LKB). The radioactivity measured refers to intact DNA, since DNA from dead cells is degraded into small fragments which are not retained by the filters. In order to determine the cytotoxicity, *i.e.* the killing of cells, the standard formula for the JAM test was used: % specific killing = (S=E)/S 100, with E = experimentally obtained DNA in the presence of effector cells (in cpm) and S = obtained DNA in the absence of effector cells (spontaneously).

It turned out that an F_V antibody construct according to the invention can activate NK cells and lyse CD30$^+$ L540CY Hodgkin's disease cells, the lysis being more intense than when bimAbHRS-3/A9 is used.

(C) Influence on murine tumors

CD30$^+$ L540CY Hodgkin's lymphomas were established in SCID mice as described by Hombach, A. *et al.*, Int. J. Cancer 55, (1993), 830; Renner, C. *et al.*, J. Hematotherapy 4, (1995), 447. To this end, $1.5x10^7$ tumor cells in 200 µl PBS were

injected subcutaneously into the right side of the mice. The tumor development, *i.e.* the diameter of the tumor, was determined twice a week. Mice suffering from tumors of 4-6 mm in diameter were divided into various groups and were given an F_v antibody construct according to the invention in 200 µl PBS together with peripheral blood lymphocytes containing NK cells (PBL cells). The tumor volume and its development were determined (*cf.* legend of figure 4).

It turned out that an F_v antibody construct according to the invention can activate NK cells both *in vitro* and *in vivo*, lysing $CD30^+$ L540CY Hodgkin's disease cells.

Claims

1. F_V antibody construct having binding sites for an CD16 receptor and a CD30 surface protein.

2. F_V antibody construct according to claim 1, wherein the CD16 receptor is derived from NK cells.

3. F_V antibody construct according to claim 1 or 2, wherein the CD30 surface protein is derived from Hodgkin's disease or Reed-Sternberg cells.

4. F_V antibody construct according to any of claims 1 to 3, wherein one binding site is present each.

5. F_V antibody construct according to claim 4, encoded by the expression vector pKID16-30 (DSM 12960).

6. F_V antibody construct according to any of claims 1 to 3, wherein two binding sites are present each.

7. Expression vector, coding for the F_V antibody construct according to any of claims 1 to 6.

8. Expression vector according to claim 7, namely pKID16-30 (DSM 12960).

9. Transformant, containing the expression vector according to claim 7 or 8.

10. A method of producing the F_V antibody construct according to any of claims 1 to 6, comprising culturing the transformant according to claim 9 under suitable conditions.

11. Kit comprising:

    (a) an F_v antibody construct according to the invention
    and/or

    (b) an expression vector according to the invention, and

    (c) common auxiliary substances, such as buffers, solvents, carriers, controls and markers,

    wherein one or more representatives of the individual components may be present.

12. Use of the F_v antibody construct according to any of claims 1 to 6 for lysis of cells expressing CD30 surface proteins.

13. Use according to claim 12, wherein the cells are tumor cells.

14. Use according to claim 13, wherein the tumor cells are Hodgkin's disease or Reed-Sternberg cells.

Abstract of the Disclosure

The present invention relates to F_V antibody constructs having binding sites for a CD16 receptor and a CD30 surface protein, wherein the F_V antibody constructs are adapted to induce a regression of Hodgkin's disease. The invention also relates to DNAs coding for such F_V antibody constructs and to a method of producing the F_V antibody constructs and their use.


XhoI
AseI
1 CTCGAGAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTAT
EcoRI
RBS
79 GCTCCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGAATTCATTAAAGAGGAGAAATTAAC
PelB leader
AlwNI
NcoI
Serum A
157 CATGAAATACCTATTGCCTACGGCAGCCGCTGGCTTGCTGCTGCTGGCAGCTCAGCCGCCATGGCGCAGGTGCAGCTG
1 MetAlaGlnValGlnLeu
VH
anti-CD16
EcoRV
235 CAGCAGTCTGGAGCTGAGCTGGTAAGGCCTGGGACTTCAGTGAAGATATCCTGCAAGGCTTCTGGCTACACCTTCACT
7 GlnGlnSerGlyAlaGluLeuValArgProGlyThrSerValLysIleSerCysLysAlaSerGlyTyrThrPheThr
CDR-H1
EcoRV
CDR-H2
313 AACTACTGGCTAGGTTGGGTAAAACAGAGGCCTGGACATGGACTCGAGTGGATTGGAGATATCTACCCTGGAGGTGGT
33 AsnTyrTrpLeuGlyTrpValLysGlnArgProGlyHisGlyLeuGluTrpIleGlyAspIleTyrProGlyGlyGly
391 TATACTAACTACAATGAGAAATTCAAGGGCAAGGCCACAGTGACTGCAGACACATCCTCCAGAACTGCCTACGTGCAG
59 TyrThrAsnTyrAsnGluLysPheLysGlyLysAlaThrValThrAlaAspThrSerSerArgThrAlaTyrValGln
CDR-H3
469 GTCAGGAGCCTGACATCTGAGGACTCTGCTGTCTATTTCTGTGCAAGATCGGCTAGCTGGTACTTCGATGTCTGGGGC
85 ValArgSerLeuThrSerGluAspSerAlaValTyrPheCysAlaArgSerAlaSerTrpTyrPheAspValTrpGly
CH1
HindIII
Linker
EcoRV
547 GCACGGACTACGGTCACCGTCTCCTCAGCCAAAACAACACCCAAGCTTGGCGGTGATATCGAGCTCACTCAGTCTCCA
111 AlaArgThrThrValThrValSerSerAlaLysThrThrProLysLeuGlyGlyAspIleGluLeuThrGlnSerPro
VL
anti-CD30
625 AAATTCATGTCCACATCAGTAGGAGACAGGGTCAACGTCACCTACAAGGCCAGTCAGAATGTGGGTACTAATGTAGCC
137 LysPheMetSerThrSerValGlyAspArgValAsnValThrTyrLysAlaSerGlnAsnValGlyThrAsnValAla
703 TGGTTTCAACAAAAACCAGGGCAATCTCCTAAAGTTCTGATTTACTCGGCATCTTACCGATACAGTGGAGTCCCTGAT
163 TrpPheGlnGlnLysProGlyGlnSerProLysValLeuIleTyrSerAlaSerTyrArgTyrSerGlyValProAsp
781 CGCTTCACAGGCAGTGGATCTGGAACAGATTTCACTCTCACCATCAGCAATGTGCAGTCTGAAGACTTGGCAGAGTAT
189 ArgPheThrGlySerGlySerGlyThrAspPheThrLeuThrIleSerAsnValGlnSerGluAspLeuAlaGluTyr
C kappa
NotI
859 TTCTGTCAGCAATATCACACCTATCCTCTCACGTTCGGAGGGGGCACCAAGCTGGAAATCAAACGGGCTGATGCTGCG
215 PheCysGlnGlnTyrHisThrTyrProLeuThrPheGlyGlyGlyThrLysLeuGluIleLysArgAlaAspAlaAla
BamHI
c-myc epitope
His6 tail
BglII
937 GCCGCTGGATCCGAACAAAAGCTGATCTCAGAAGAAGACCTAAACTCACATCACCATCACCATCACTAAAGATCTATT
241 AlaAlaGlySerGluGlnLysLeuIleSerGluGluAspLeuAsnSerHisHisHisHisHisHis
RBS
Pel B leader
NcoI
1015 AAAGAGGAGAAATTAACCATGAAATACCTATTGCCTACGGCAGCCGCTGGCTTGCTGCTGCTGGCAGCTCAGCCGGCC
NcoI
Serum A
VH
anti-CD30
1093 ATGGCGGCCATGGCCCAGGTGCAACTGCAGCAGTCAGGGGCTGAGCTGGCTAGACCTGGGGCTTCAGTGAAGATGTCC
1 MetAlaGlnValGlnLeuGlnGlnSerGlyAlaGluLeuAlaArgProGlyAlaSerValLysMetSer
1171 TGCAAGGCTTCTGGCTACACCTTTACTACCTACACAATACACTGGGTAAGACAGAGGCCTGGACACGATCTGGAATGG
24 CysLysAlaSerGlyTyrThrPheThrThrTyrThrIleHisTrpValArgGlnArgProGlyHisAspLeuGluTrp
1249 ATTGGATACATTAATCCTAGCAGTGGATATTCTGACTACAATCAGAACTTCAAGGGCAAGACCACATTGACTGCAGAC
50 IleGlyTyrIleAsnProSerSerGlyTyrSerAspTyrAsnGlnAsnPheLysGlyLysThrThrLeuThrAlaAsp
1327 AAGTCCTCCAACACAGCCTACATGCAACTGAACAGCCTGACATCTGAGGACTCTGCGGTCTATTACTGTGCAAGAAGA
76 LysSerSerAsnThrAlaTyrMetGlnLeuAsnSerLeuThrSerGluAspSerAlaValTyrTyrCysAlaArgArg
CH1
1405 GCGGACTATGGTAACTACGAATATACCTGGTTTGCTTACTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGCCAAA
102 AlaAspTyrGlyAsnTyrGluTyrThrTrpPheAlaTyrTrpGlyGlnGlyThrThrValThrValSerSerAlaLys
HindIII
Linker
EcoRV
VL
anti-CD16
1483 ACAACACCCAAGCTTGGCGGTGATATCCAGGCTGTTGTGACTCAGGAATCTGCACTCACCACATCACCTGGTGAAAC
128 ThrThrProLysLeuGlyGlyAspIleGlnAlaValValThrGlnGluSerAlaLeuThrThrSerProGlyGluTh
CDR-L1
1560 AGTCACACTCACTTGTCGCTCAAATACTGGGACTGTTACAACTAGTAACTATGCCAACTGGGTCCAAGAAAAACCAGA
153 rValThrLeuThrCysArgSerAsnThrGlyThrValThrThrSerAsnTyrAlaAsnTrpValGlnGluLysProAs
CDR-L2
1638 TCATTTATTCACTGGTCTAATAGGTCATACCAACAACCGAGCTCCAGGTGTTCCTGCCAGATTCTCAGGCTCCCTGAT
179 pHisLeuPheThrGlyLeuIleGlyHisThrAsnAsnArgAlaProGlyValProAlaArgPheSerGlySerLeuIl
CDR-L3
1716 TGGAGACAAGGCTGCCCTCACCATCACAGGGGCACAGACTGAGGATGAGGCAATATATTTCTGTGCTCTATGGTATAA
205 eGlyAspLysAlaAlaLeuThrIleThrGlyAlaGlnThrGluAspGluAlaIleTyrPheCysAlaLeuTrpTyrAs
NotI
BamHI
1794 CAACCATTGGGTGTTCGGTGGAGGAACCAAACTGACTGTCCTAGGCCAGCCCAAGTCTGCGGCCGCTGGATCCGAACA
231 nAsnHisTrpValPheGlyGlyGlyThrLysLeuThrValLeuGlyGlnProLysSerAlaAlaAlaGlySerGluGl

Fig. 1

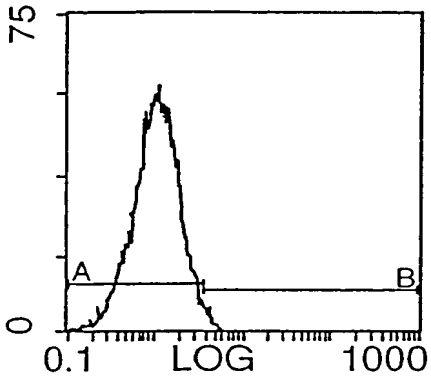

c-myc epitope
His6 tail
XbaI
BclI
NheI
1872 AAAGCTGATCTCAGAAGAAGACCTAAACTCACATCACCATCACCATCACTAATCTAGAGGCCTGTGCTAATGATCAGC
257 nLysLeuIleSerGluGluAspLeuAsnSerHisHisHisHisHisHis
HpaI
1950 TAGCTTGAGGCATCAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTTAAC
SalI
EarI
PvuI
FspI
BglI
2028 GTCGACCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGGA
2106 CGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCT
NaeI
2184 AGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGG
f1 IR
DraIII
2262 GCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAG
2340 TGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCA
2418 AACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTT
SspI
2496 AAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGCTTACAATTTAGGTGGCACTTTT
BspHI
2574 CGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAA
SspI
EarI
2652 CCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTT
ApaL
2730 TTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGT
XmnI
2808 GCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCA
DraI
2886 ATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGC
ScaI
1000
2964 CGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTA
β-Lactamase
PvuI
3042 AGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCG
3120 AAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAA
FspI
3198 GCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAA
AseI
3276 CTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCG
BglI
BsaI
3354 GCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTG
3432 GGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGA
3510 CAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATT
DraI
DraI
BspHI
3588 GATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAA
3666 CGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGC
3744 GTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTT
3822 TTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCAC
AlwNI
3900 TTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAG
ColE1
2000
ApaLI
3978 TCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGC
4056 ACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTT
4134 CCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGG
4212 GGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCA
4290 GGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCAC
4368 ATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGC
EarI
4446 AGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCG
AseI
BspMI
4524 CGTTGGCCGATTCATTAATGCAGGTATCACGAGGCCCTTTCGTCTTCAC

Fig. 1 (cont'd)

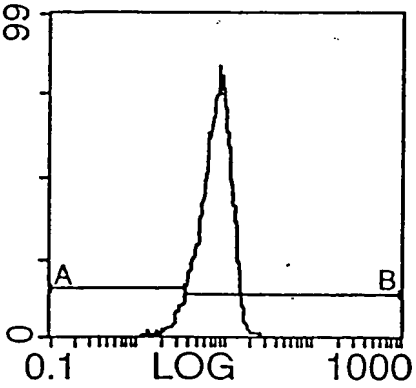
Fig. 2

Granulocytes ($CD16^+$)

Neg. Control

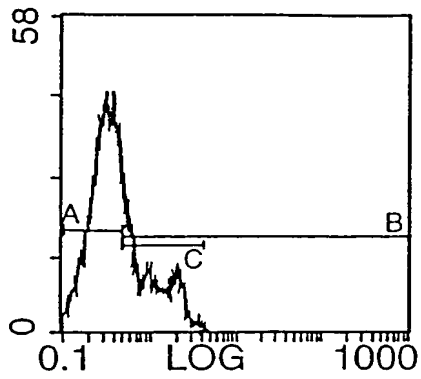


F_v antibody construct according to the invention

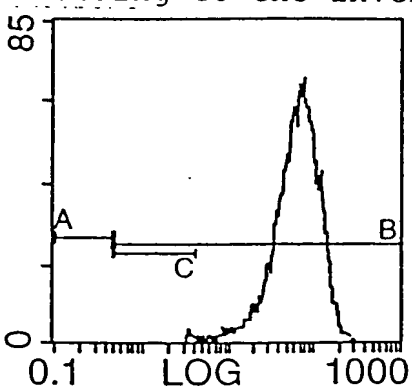


L540CY cells ($CD30^+$)

Neg.Control



F_v antibody construct according to the invention



Fluorescence Intensity

Fig. 3

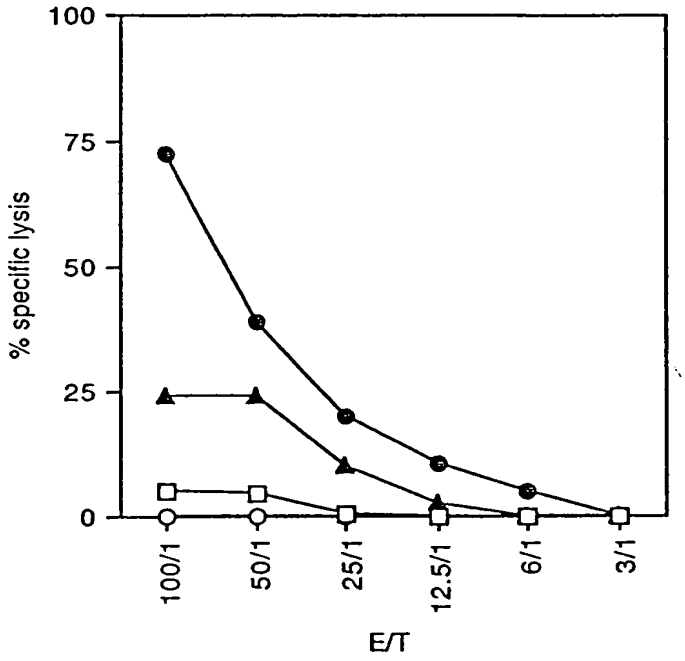


Fig. 4